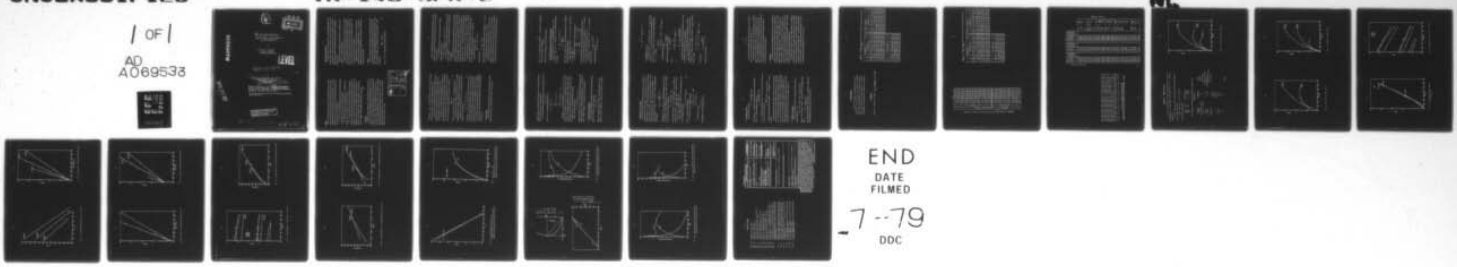
AD-A069 533 PRINCETON UNIV N J DEPT OF STATISTICS F/G 6/1
LIVER REDOX RESISTANCE, A DYNAMIC MODEL OF GLUCONEOGENIC LACTAT--ETC(U)
MAR 79 A T JAMES N00014-75-C-0453
UNCLASSIFIED TR-146-SFR-2 NL

| OF |
AD
A069533



END
DATE
FILMED
7-79
DDC

LIVER REDOX RESISTANCE, A Dynamic Model of Gluconeogenic Lactate Metabolism.

by

Alan T. James*
Princeton University

TR-146-SER-2

Technical Report, No. 146, Series 2
Department of Statistics
Princeton University
March 1979
24p.

Research supported in part by a contract with the Office of Naval Research, No. N00014-75-C-0453, awarded to the Department of Statistics, Princeton University, Princeton, New Jersey.

*On leave from The University of Adelaide, Adelaide, South Australia.

Summary

For isolated hepatocytes from starved rats metabolising lactate, the time curves of pyruvate and glucose produced and lactate remaining are fitted with elementary mathematical functions by simple statistical procedures. The fitted functions are very close approximations to the solutions of differential equations which express the following model:

(i) The rate of net pyruvate production, which involves hydrogen disposal, is proportional to the rise of cytosolic redox potential.

(ii) Glucose is produced at a rate proportional to the concentration of pyruvate, the initial substrate in the chain of reactions.

(iii) The lactate remaining is that part not transformed into pyruvate or glucose less a small amount progressively transformed into other substances or catabolised. Since the rise of redox potential is measured in volts and the net hydrogen flux from the lactate can be expressed in amps of electron flow involved, the ratio in ohms is called a redox resistance. The estimated flux curves for hydrogen and gluconeogenic intermediates are shown.

1. Experimental

Lactate was added to suspensions of isolated hepatocytes from starved rats to make up 2 ml solutions of approximate concentrations 10 mM Lac, 5 mM Lac, and 2.5 mM Lac. After incubations during times up to an hour, pyruvate, glucose and lactate were assayed. The data are from unpublished results of Berry et al. (M. N. Berry, personal communication). Details of cell preparation, incubations and analyses have been published elsewhere (Berry and Kun, 1972). The results are given under the heading "obs." in Tables 1A, 1B and 1C and as the plotted points in Figures 1A, 1B, 1C, 2 and 3.

2. Introduction to the Analysis

The aim of the paper is to develop and fit a simple mathematical model, perhaps the simplest, which will fit the data to within its order of accuracy. Such a model supplies a very precise statistical analysis and summary of the data in much sharper focus than crude averages and yields estimates of instantaneous metabolic fluxes.

Improved data will undoubtedly require modification of the model, either refinement or its complete replacement.

Furthermore, while the model, together with the data which it fits, establishes certain quantitative relations between the variables with considerable precision and certainty under the conditions of experiments, the relations may be open to interpretations other than the ones that suggested the model. This is particularly true of causal interpretations.

A mathematical model of this "simplicity" may well fit other situations, unrelated except for the abstract mathematical relations. It illustrates the mathematical and statistical problems which can arise from even a simple model and how they can be solved.

3. Model

Net pyruvate production requires disposal of the hydrogen from the lactate. It was conjectured that the rate would depend upon the lactate pyruvate ratio. The data indicated something approximating a logarithmic relation, which, according to the Nernst equation, can be interpreted as the redox potential. Hence, we postulate:

$$\frac{dP}{dt} = \alpha \ln \frac{L/P}{\kappa} , \qquad (1)$$

where L and P are the amounts of lactate and pyruvate.

The constants α and κ have to be estimated. The latter is the "equilibrium" value of L/P of about 7, at which net pyruvate production ceases.

The slight delay in gluconeogenesis and subsequently increasing rate suggested that the rate of glucose production might depend upon pyruvate levels, because carboxylation of pyruvate is the first reaction in the chain.

An attempt to fit a Michaelis Menton relation between rate of glucose production and pyruvate gave a large, rather indeterminate value of the MM constant Km. Since large values of Km were consistent with the data, it was decided to take Km as infinity, thereby simplifying the relation to a linear one,

$$\frac{dG}{dt} = \beta P \qquad (2)$$

which, in fact, suffices to fit the data. G is the amount of glucose.

Since higher initial levels of lactate yield lower values of β, this simple relationship may well be regarded an approximation to a more complex situation.

Nevertheless, the fit to glucose as well as to the pyruvate can be used to calculate the residual lactate,

$$L = L_0 - P - 2(G - G_0) - \gamma t \ . \qquad (3)$$

The constants L_0 and G_0 are the initial lactate and glucose and, of course, two molecules of lactate make one molecule of glucose. The last term allows for a slight but significant amount of carbon unaccounted for by the pyruvate and glucose, which for simplicity is taken as proportional to time with a constant γ to be estimated. Some carbon may be catabolised, but, rather, the unaccounted carbon may go into the formation of other substrates, particularly replenishment of the Krebs cycle.

4. Fitting the Model

For prescribed initial values and constants, the three equations must determine the three variables as unique functions of time, P(t), G(t), L(t).

However, although the model may be considered unduly parsimonious, a direct attempt to fit the relations by estimating the constants will run into three very serious difficulties:

(i) The differential equations are nonlinear and hence will require numerical methods of solution.

(ii) There is a singularity at $t = 0$, viz., $L/P = \infty$.

(iii) The functions obtained as solutions are nonlinear functions of the constants or parameters, i.e., we have nonlinear parameters which will require iterative estimation.

Iterative estimation of parameters using numerical solution of differential equations is a complex operation that could easily go awry, and there are reasons why it might; namely, biological data involving intact cells is subject to a considerable coefficient of variation, in this case about 8%, and has occasional aberrant values or outliers. In addition, the model needs to be checked against the data from stage to stage as it is being fitted to see that it is appropriate.

The problems were bypassed by Dr. A. R. Grivell's discovery of a further empirical relation approximately numerically consistent with the model, namely, $\ln(L/P)$ is approximately linearly related to $\ln t$. See Figure 4.

Substitution in equation (1) yields

$$\frac{dP}{dt} = \beta_1 + \beta_2(1 + \ln t) \ ,$$

which integrates to

$$P = \beta_1 t + \beta_2 t \ln t \ .$$

Hence, pyruvate can now be fitted by multiple linear regression of P on t and $t \ln t$, yielding the fitted curves shown in Figures 1A, 1B and 1C.

During the second half hour, the pyruvate curve for 2.5 mM Lac has fallen steadily. As the model will not fit this part of the curve, the fit has been truncated in this case shortly after the maximum. The reason for the restriction of the model to high redox states is that some completely different regulatory

mechanisms appear to be involved for low redox states of the cytosol when hydrogen has to be shuttled out of the mitochondrion.

The second differential equation for glucose can now be integrated to yield

$$G = G_0 + \beta I(t)$$

where $I(t)$ is the integrated pyruvate given by

$$\begin{aligned} I(t) &= \int_0^t P(\tau)\, d\tau \\ &= \int_0^t \beta_1 \tau + \beta_2 \tau \ln \tau \, d\tau \\ &= \tfrac{1}{2}\beta_1 t^2 + \tfrac{1}{2}\beta_2 t^2 (\ln t - \tfrac{1}{2}) \ . \end{aligned}$$

The estimates b_1, b_2 can be substituted for β_1, β_2 and G_0 and β estimated by linear regression of G on $I(t)$ yielding estimates $\hat{G}_0$, b^*, shown in Table 2, and fits shown in Figures 5A, 5B, and 5C. Where different initial concentrations of lactate have been used, the slopes β prove to be different, but a common value $\hat{G}_0$ is fitted using concurrent regression.

Finally, from equation (3) we have

$$L + P + 2(G - G_0) = L_0 - \gamma t \ .$$

Hence we can estimate L_0 and γ by substituting the observed lactate and fitted pyruvate and glucose in the lefthand side and regressing on t; see Figure 6 for plots of lefthand side against t and Figure 3 for the fitted lactate.

5. Checks upon the Approximation

How closely does our approximate solution satisfy the original differential equations? The answer is given by calculating

$$P' = b_1 + b_2(1 + \ln t)$$

from our fit and plotting against $\ln(L/P)$ also calculated from our fit and seeing if it yields a straight line. Figures 7A, 7B, 7C show it is very good

when $P' > 0$. The slope yields an estimate of α and the point of intersection with the abscissa yields an estimate of

$$\eta = \ln \kappa \ .$$

A more critical verification is given in the next section.

6. Exact Numerical Solution of the Differential Equations

One can use the fact that $x = L$ is approximately linear in t and $y = \ln(L/P)$ is linear in $\ln t$. In terms of x and y, the differential equations become

$$x' = -\alpha(y - \eta) - 2\beta P(x,y) - \gamma$$

$$y' = \frac{x'}{x} - \frac{\alpha(y - \eta)}{P(x,y)}$$

where $P(x,y) = x \exp(-y)$, and x' may be calculated from the first differential equation and substituted in the second.

A numerical solution uses steps of Δt for x and $\Delta \ln t = \ln((t+\Delta t)/t)$ for y, since $\frac{dy}{d\ln t} = ty'$.

For the 10 mM data of experiment 1 for which the estimates are

α : $a = .03303$ $\quad\quad$ γ : $c = .0133$

β : $b = .030191$ $\quad\quad$ η : $e = 1.854$

the differential equation was solved using the fitted regression values at $t = 30$ as initial values because there is a singularity at $t = 0$. The results of the solution are shown in Table 3 and Figures 8, 9, 10.

If we assume x' is exactly linear in t and y' in $\ln t$, we obtain a relation of the form

$$P = \beta_3 t^{\rho_1} + \beta_4 t^{1+\rho_1}$$

which agrees almost exactly with

$$P = \beta_1 t + \beta_2 t \ln t \ .$$

7. Interpretation of the Constants

Some care is worthwhile in the choice of the functions of the constants or parameters which enter into the model. Initially, one uses β_1, β_2 for the pyruvate because it is a simple linear function of them and they can be estimated by linear regression. However, β_1 is not very readily interpreted, as its complicated transformation under change of scale indicates (see Appendix 1). Instead, the maximal value t_{max1} of t at which P reaches its maximum seems more meaningful:

$$t_{max1} = \exp\left(-\frac{\beta_1}{\beta_2} - 1\right) .$$

The maximum value $P_{max} = P(t_{max1})$ is a meaningful parameter to go with it, but as its estimate is strongly correlated with t_{max1}, instead, we use the average rate of increase of pyruvate to the maximum

$$P_{av}' = P_{max}/t_{max1} = -\beta_2$$

as illustrated in Figure 11. Table 2 gives estimates of t_{max1} expressed in liver grm. wet wt. mins. as $t^*_{max1} = wt_{max1}$ where w = liver dry wt. in grms. $\times$ 3·77, and of P'_{av} in μ mols/grm. wet wt./min.

In terms of these parameters, the model, or rather the approximation to it, has a more meaningful expression

$$P = P_{av}' \; t \; \ln (e \; t_{max1}/t) \qquad (4)$$

where e is base of natural logarithms.

We can then write for the integrated pyruvate

$$I = \frac{1}{2} t^2\left(\beta_1 + \beta_2\left(\ln t - \tfrac{1}{2}\right)\right)$$
$$= \frac{1}{2} t\left(P + \tfrac{1}{2}P_{av}' \; t\right) .$$

Since glucose is given by

$$G = G_o + \beta I ,$$

on substitution in the lactate equation we have

$$L = L_o - (\beta t + 1)P - \tfrac{1}{2}P_{av}' \; \beta t^2 - \gamma t .$$

8. Calculation of κ and α

We need them in terms of L_o, t_{max1}, P_{av}', β and γ. If $L_{max} = L(t_{max1})$ is the value of L at t_{max1} (not to be confused with the maximum value of L which is, of course, the initial value L_o), then the normal lactate/pyruvate ratio, κ, is given by

$$\kappa = L_{max}/P_{max}$$
$$= \left((L_o/t_{max1}) - \gamma\right)/P_{av}' - (3/2) \; \beta \; t_{max1} - 1 .$$

Differentiating (4), we have

$$P' = P_{av}' \; \ln(t_{max1}/t) .$$

Combining this with the differential equation

$$P' = \alpha \ln \frac{(L/P)}{\kappa}$$

yields

$$\ln \frac{(L/P)}{\kappa} = \frac{P_{av}'}{\alpha} \ln(t_{max1}/t) ,$$

i.e.,

$$\alpha = P_{av}' \; \ln(t_{max1}/t) \Big/ \ln\left(\frac{(L/P)}{\kappa}\right) .$$

The constancy of the righthand side follows from the combination of the original differential equation model with our approximation.

At $t = t_{max1}$ the righthand side becomes indeterminate as its numerator and denominator both vanish, and likewise at $t = 0$, they both become infinite. To evaluate α we choose a value in between. The value $t_1 = t_{max1}/e$ seems convenient. Putting

$$P_1 = P(t_1) = 2 \; P_{av}' \; t_1$$
$$L_1 = L(t_1) = L_o - \gamma t_1 - \left(\tfrac{5}{4} \beta t_1 + 1\right) P_1 ,$$

we can calculate

$$\alpha = P_{av}' / \ln\left(\frac{L_1/P_1}{\kappa}\right) .$$

Estimates are given in Table 2.

9. Liver Redox Resistance

By using the Nernst equation

$$\Delta E = .013 \ln\left(\frac{L/P}{\kappa}\right) \text{ volts}$$

and expressing P in coulombs of reducing electrons that have been shed by L using $2F = 193000$ coulombs/mol, we can write

$$P' = \frac{.013}{\rho} \ln\left(\frac{L/P}{\kappa}\right) = \frac{\Delta E}{\rho} \quad .$$

If P' is expressed in coulombs/sec, then ρ will be in ohms. Hence we can write ρ, the liver redox resistance, as

$$\rho = \frac{.013}{\alpha} \text{ ohm gram liver wet weight} \quad .$$

It comes to about 8 ohm gram liver wet weight. ρ is estimated by r^*, as given in Table 2.

An 8 ohm gram wet weight redox resistance implies a shuttle of one milliamp of reducing equivalents per gram wet weight per 8 millivolts rise of redox potential. Since a milliamp corresponds to approximately 0.3 μMH_2/min, it would take a rise of 27 mV in the redox potential to shuttle one μ mol H_2/min/gram wet weight. The relation is only asserted under the conditions of the experiment, which include an adequate oxygen supply and no exogenous lipids or other source of reducing equivalents other than the high lactate, no exogenous ammonia, etc.

10. Metabolic Flux Dynamics

The flux of the 3-carbon precursors of glucose must be twice the rate of glucose production, G', i.e.,

$$2G' = 2\beta P = 2\beta^* P_{av}' \, wt\left(1 + \ln\left(t^*_{max1}/(wt)\right)\right)$$

where w = liver dry weight in grams × 3.77, and the flux of the hydrogen shuttle must be

$$P' = P_{av}' \ln\left(t^*_{max1}/(wt)\right) \quad ,$$

both fluxes being in mols/grm. liver wet wt/min. The aspartate transport out of the mitochondrion due to both processes will be their sum

$$2G' + P' \quad .$$

The fluxes may be estimated by substituting the estimates of the constants given in Table 2 and are shown in Figures 12A, 12B, 12C.

The estimates of the fluxes are only first order approximations in that they would not be accurate enough to determine the slow change over the hour of the amount of an intermediate metabolite as the difference between the influx and efflux. Nevertheless, from the fluxes, it may be possible to infer the time curves of some substrate concentrations by enzyme kinetics and thereby of others by equilibrium constants and redox potentials.

11. Discussion

The fitting of time curves by elementary statistical means, as exemplified in the paper, supplies a more accurate and efficient extraction of information than curves drawn by eye, allows a critical comparison between samples, and, in future, comparison between treatments and controls. It yields interpolated values for times not observed. Integrals can be calculated to relate to accumulations, e.g., of glucose, and derivatives yield instantaneous fluxes. The enhanced precision of the analysis should help to unravel the complicated phenomena of metabolic dynamics and perhaps discriminate between conflicting theories.

The elementary fitted functions are close to approximations to the solutions of differential equations, based on a model which relates the flux of the hydrogen shuttle to the cytosolic redox potential. Under the conditions of these particular experiments, they appear to be proportional. The constant of proportionality is the redox resistance, a perhaps important physiological parameter. It would be interesting to know if pretreatment of the rat can alter it.

ACKNOWLEDGEMENT

The data in this paper are unpublished results of Professor M. N. Berry and Dr. A. R. Grivell of the Department of Clinical Biochemistry, Flinders Medical Center, The Flinders University of South Australia. The author acknowledges with gratitude their permission to quote it.

REFERENCES

Berry, M. N. and Kun, E. (1972). Eur. J. Biochem., 27, 395-400.

TABLE 1A. Observed and Fitted Values of Pyruvate, Glucose and Lactate in μ mols.

EXPERIMENT No. 1 - Liver Dry Weight 0.01685 g.

t	10 mM. Lac.						5 mM. Lac.					
	P		G		L		P		G		L	
min.	obs.	fit.	obs.	fit.	obs.	fit.	obs.	fit.	obs.	fit.	obs.	fit.
0	0.01	0	0.078	0.100	18.2	18.22	0.01	0	0.071	0.100	8.75	9.05
5	0.44	0.425	0.16	0.137	17.55	17.65	0.36	0.355	0.17	0.149	8.56	8.55
15	0.89	0.891	0.35	0.341	16.82	16.64	0.64	0.671	0.40	0.400	7.87	7.66
30	1.29	1.298	0.86	0.846	15.12	15.03	0.87	0.842	0.98	0.948	6.52	6.27
60	1.62	1.627	2.2	2.207	11.36	11.58	0.68	0.687	2.07	2.084	3.72	3.91

	2.5 mM. Lac.					
t	P		G		L	
0	0.01	0	0.075	0.100	4.8	4.81
5	0.27	0.296	0.18	0.162	4.37	4.39
15	0.50	0.478	0.41	0.443	3.67	3.64
30	0.43	0.437	0.95	0.938	2.67	2.68

TABLE 1B. Observed and Fitted Values of Pyruvate, Glucose, and Lactate in μ mols.

EXPERIMENT No. 2 - Liver Dry Weight 0.01725 g.																							
	10 mM. Lac.								5 mM. Lac.														
t	P		G		L		P		G		L		P		G		L		P		G		L	
min.	obs.	fit.	obs.	fit.	obs.	fit.	obs.	fit.	obs.	fit.	obs.	fit.	obs.	fit.	obs.	fit.	obs.	fit.	obs.	fit.	obs.	fit.	obs.	fit.
0	0.022	0	0.12	0.150	21.23	21.15	0.027	0.02	0.150	0.120	11.346	10.714	11.07	0.109	0.120	0.150	11.346	10.714	11.07	0.109	0.120	0.150	11.346	10.714	11.07
5	0.44	0.333	0.227	0.181	19.94	20.61	0.45	0.335	0.315	0.227	0.323	0.196	9.81	10.68	10.58	0.227	0.323	0.196	9.81	10.68	10.58	0.227	0.323	0.196	9.81	10.68	10.58
15	0.718	0.747	0.42	0.369	19.45	19.49	0.694	0.523	0.612	0.444	0.46	0.437	10.01	9.97	9.62	0.444	0.46	0.437	10.01	9.97	9.62	0.444	0.46	0.437	10.01	9.97	9.62
30	1.14	1.175	0.815	0.864	18.69	17.57	0.82	0.704	0.804	0.901	0.835	0.983	7.67	8.72	8.06	0.901	0.835	0.983	7.67	8.72	8.06	0.901	0.835	0.983	7.67	8.72	8.06
60	1.73	1.714	2.37	2.360	12.25	13.05	0.781	0.784	0.767	2.28	2.24	2.217	4.86	5.01	5.07	2.28	2.24	2.217	4.86	5.01	5.07	2.28	2.24	2.217	4.86	5.01	5.07

TABLE 1C. Observed and Fitted Values of Pyruvate, Glucose and Lactate in μ mols.

EXPERIMENT No. 3 - Liver Dry Weight 0.01638 g.																							
	10 mM. Lac.								5 mM. Lac.														
t	P		G		L		P		G		L		P		G		L		P		G		L	
min.	obs.	fit.	obs.	fit.	obs.	fit.	obs.	fit.	obs.	fit.	obs.	fit.	obs.	fit.	obs.	fit.	obs.	fit.	obs.	fit.	obs.	fit.	obs.	fit.
0	0.016	0	0.175	0.389	19.21	21.56	0.016	0	0.181	0.389	10.5	10.69	0.016	0	0.181	0.389	10.5	10.69	0.016	0	0.181	0.389	10.5	10.69
5	0.37	0.418	0.33	0.418	21.44	20.94	0.41	0.318	0.36	0.481	10.46	10.28	0.41	0.318	0.36	0.481	10.46	10.28	0.41	0.318	0.36	0.481	10.46	10.28
15	0.827	0.885	0.62	0.582	19.08	19.85	0.604	0.646	0.65	0.595	9.37	9.51	0.604	0.646	0.65	0.595	9.37	9.51	0.604	0.646	0.65	0.595	9.37	9.51
30	1.42	1.307	1.08	0.991	18.46	18.16	0.78	0.900	1.06	1.009	8.49	8.29	0.78	0.900	1.06	1.009	8.49	8.29	0.78	0.900	1.06	1.009	8.49	8.29
45	1.51	1.553	1.54	1.520	16.27	16.42	1.18	1.008	1.65	1.513	7.166	7.03	1.18	1.008	1.65	1.513	7.166	7.03	1.18	1.008	1.65	1.513	7.166	7.03
60	1.68	1.686	2.07	2.117	14.76	14.65	0.953	1.019	1.93	2.048	5.63	5.81	0.953	1.019	1.93	2.048	5.63	5.81	0.953	1.019	1.93	2.048	5.63	5.81

2.5 mM. Lac.					
t	P		G		L	
0	0.017	0	0.183	0.389	6.22	6.22
5	0.336	0.309	0.39	0.442	5.99	5.70
15	0.472	0.494	0.633	0.681	4.28	4.77
30	0.454	0.446	1.12	1.096	3.78	3.59

TABLE 2. Constants

	Initial Lactate L_o μ mols/ 2 mls.	Time to Max. P $t^*_{maxl.}$ liver grm. wet wt. mins.	Av. Rate of increase of P to Max. $P'_{av.}$ μ mols/gm. wet wt./min.	Reg. of Glucose on Int. Pyr. b* /gm. wet wt. /min.	Rate of Carbon "Loss" C* μ mols/gm. wet wt./min.	Equilib. L/P k	Redox Resistance r* ohm grm. wet wt.
Experiment 1 (Liver dry wt. 0.01685 gm.)							
10 mM Lac.	18.21 ± .28	4.49 ± .77	0.367 ± .040	0.475 ± .018	0.26 ± .14	6.21 ± .71	7.92 ± 1.12
5 mM Lac.	9.05 ± .28	2.26 ± .38	0.378 ± .040	0.740 ± .030	0.13± .14	6.76 ± .72	6.56 ± .80
2.5 mM Lac.	4.82 ± .33	1.26 ± .25	0.392 ± .092	1.085 ± .108	0.10 ± .30	6.69±1.30	5.70 ± 1.60
Experiment 2 (Liver dry wt. 0.01725 gm.)							
10 mM Lac.	21.17 ± .28	9.29±3.28	0.235 ± .039	0.521 ± .019	0.51± .14	3.98[x]	14.52[x]
5 mM Lac.	11.08 ± .20	2.70 ± .32	0.311 ± .028	0.771 ± .021	0.29 ± .10	8.16 ± .68	8.36 ± .94
Experiment 3 Liver dry wt. 0.01638 gm.)							
10 mM Lac.	21.56 ± .37	4.81 ± .83	0.361 ± .041	0.396 ± .020	0.48 ± .16	7.25 ± .71	7.65 ± 1.14
5 mM Lac.	10.71 ± .28	3.37 ± .54	0.304 ± .041	0.565 ± .029	0.15 ± .14	6.10 ± .72	9.08 ± 1.11
2.5 mM Lac.	6.24 ± .33	1.20 ± .22	0.424 ± .095	0.910 ± .119	0.43 ± .31	8.58 ±1.30	5.09 ± 1.61

x - estimated graphically

TABLE 3. Observed, Regression Fitted, and Differential Equation Solution values for Experiment No. 1, 10 mM Lac. Pyruvate, Glucose, and Lactate in μ mols.

t	P			G			L		
min.	obs.	reg.	d.e.	obs.	reg.	d.e.	obs.	reg.	d.e.
0	0.01	0	0	0.078	0.1000	0.1000	18.2	18.216	18.196
5	0.44	0.4250	0.4485	0.16	0.1365	0.1180	17.55	17.651	17.645
15	0.89	0.8911	0.9009	0.35	0.3413	0.3278	16.82	16.643	16.640
30	1.29	1.2979	1.2979	0.86	0.8460	0.8361	15.12	15.027	15.027
60	1.63	1.6270	1.6231	2.2	2.2065	2.1924	11.36	11.578	11.590

APPENDIX 1 - CHANGE OF SCALE

If w is the wet weight of liver in grams (taken as 3.77 times dry weight), we can introduce an operational time $t^* = wt$ gram wet weight mins.

Let b_1^* and b_2^* be the regression coefficients on operational time. Then we must have

$$P = b_1^* t^* + b_2^* t^* \log t^* = b_1 t + b_2 t \log t ,$$

from which we deduce that

$$\begin{bmatrix} b_1^* \\ b_2^* \end{bmatrix} = \begin{bmatrix} w^{-1} & -w^{-1}\log w \\ 0 & w^{-1} \end{bmatrix} \begin{bmatrix} b_1 \\ b_2 \end{bmatrix} .$$

The multipliers for other scale changes are given by the Dimensions.

Quantity	Dimension	Type
L, L_0, P, P_{max}, G, G_0	M	amount
1 wet wt., 1 dry wt.	L	liver weight
t, $t_{maxl.}$	T	chronological time
t^*, $t^*_{maxl.}$	$LT = T^*$	operational time = liver wet wt.×time
$\log_{10}$, ln, n	B	base of logs
α	$MT^{-1}B^{-1}$	
α^*	$MT^{*-1}B^{-1}$	
P'_{av}, β_2^*	MT^{*-1}	
β	T^{-1}	
β^*	T^{*-1}	
κ	1	number

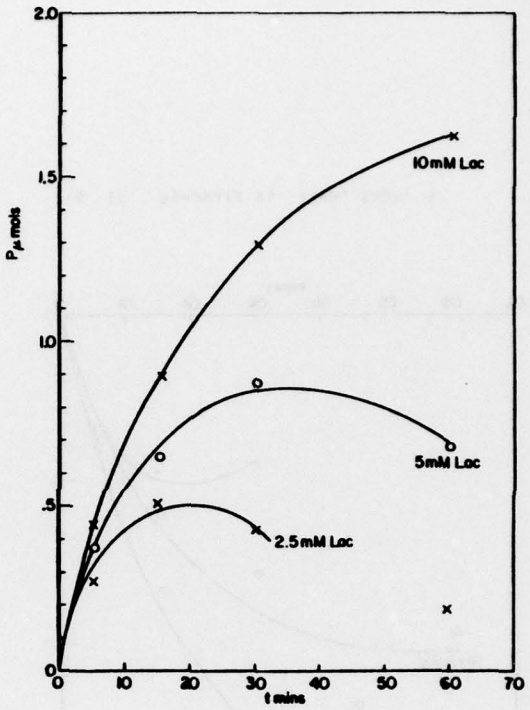


Fig. 1A. Pyruvate vs. Time, Expt. 1

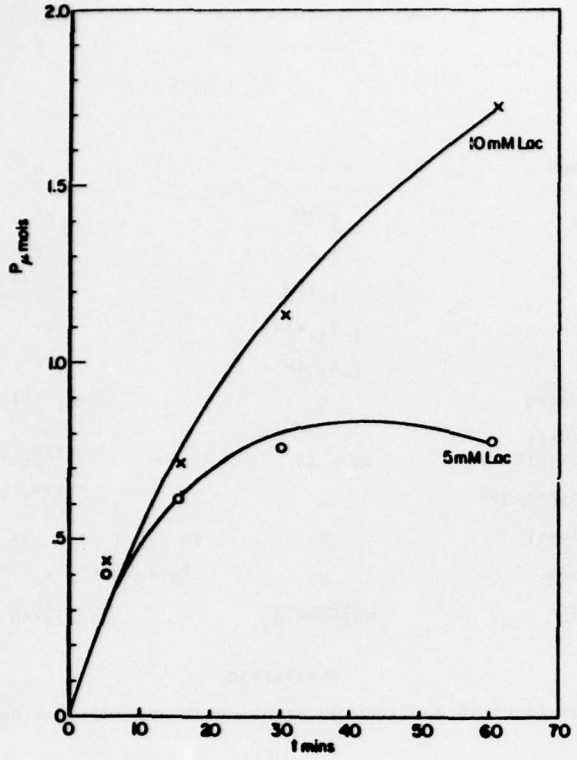


Fig. 1B. Pyruvate vs. Time, Expt. 2

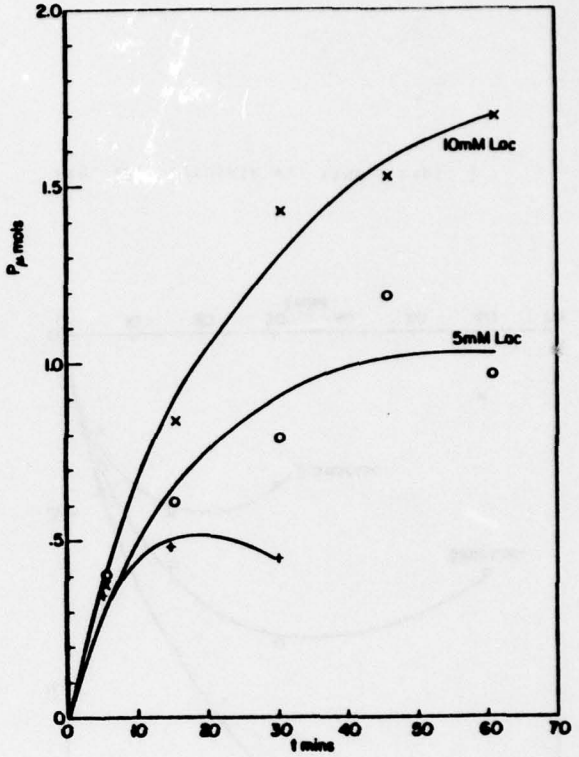


Fig. 1C. Pyruvate vs. Time, Expt. 3

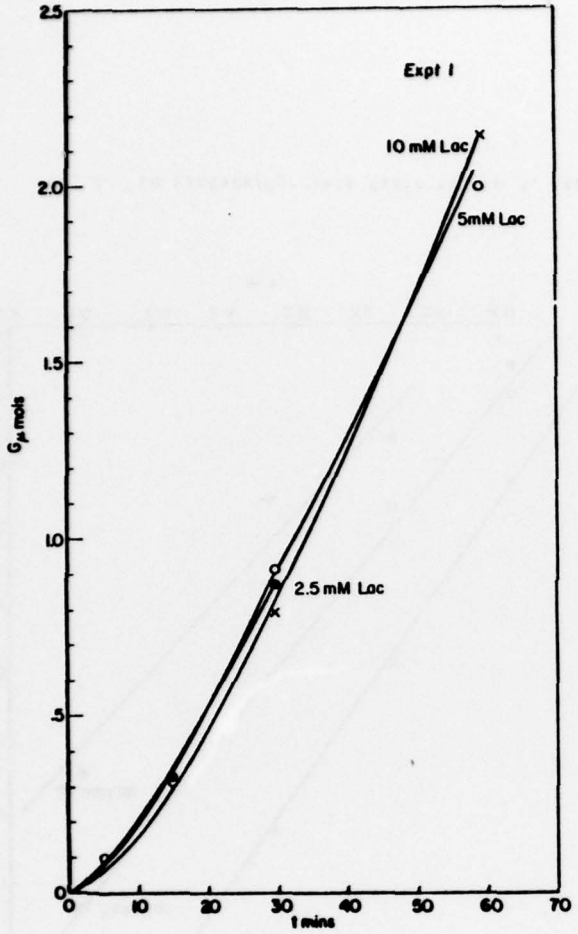


Fig. 2. Glucose minus Initial Glucose vs. Time, Expt. 1

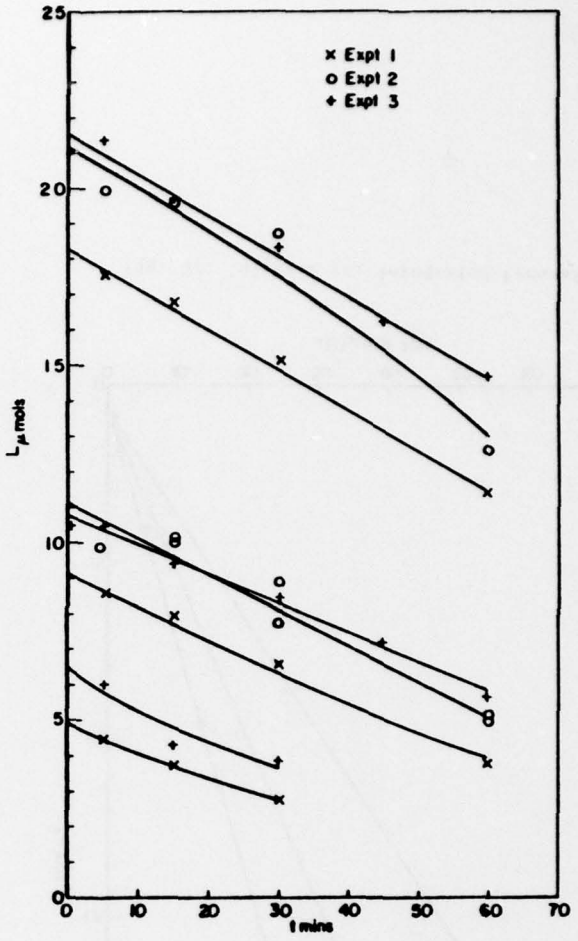


Fig. 3. Lactate vs. Time, Expts. 1, 2, and 3

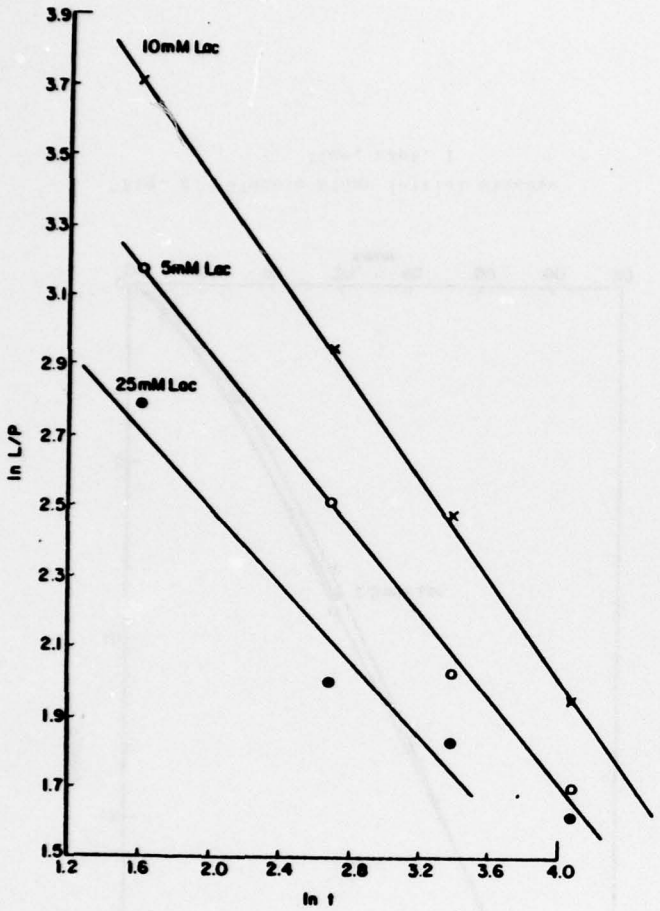


Fig. 4. Ln Lactate/Pyruvate Ratio vs. Ln t, Expt. 1

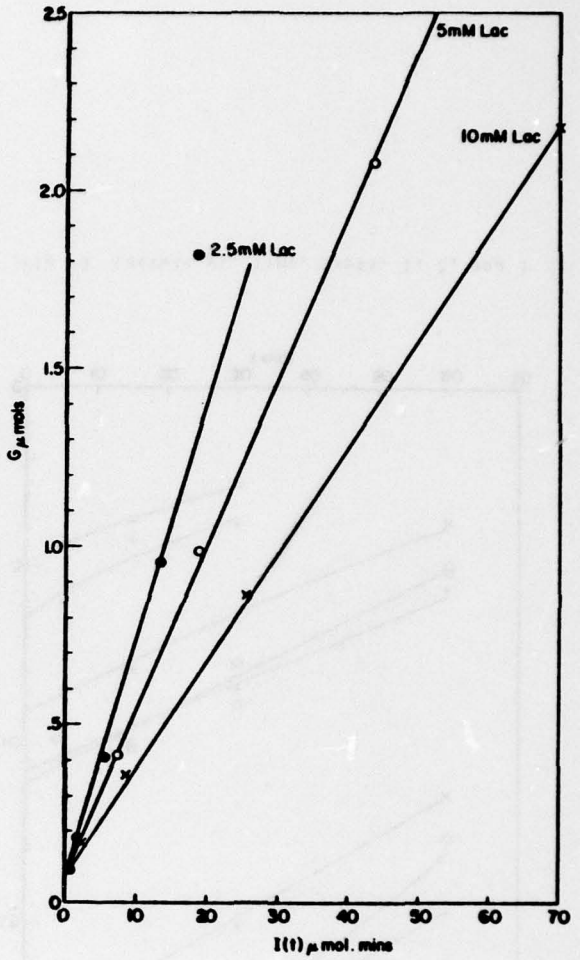


Fig. 5A. Glucose vs. Integrated Pyruvate, Expt. 1

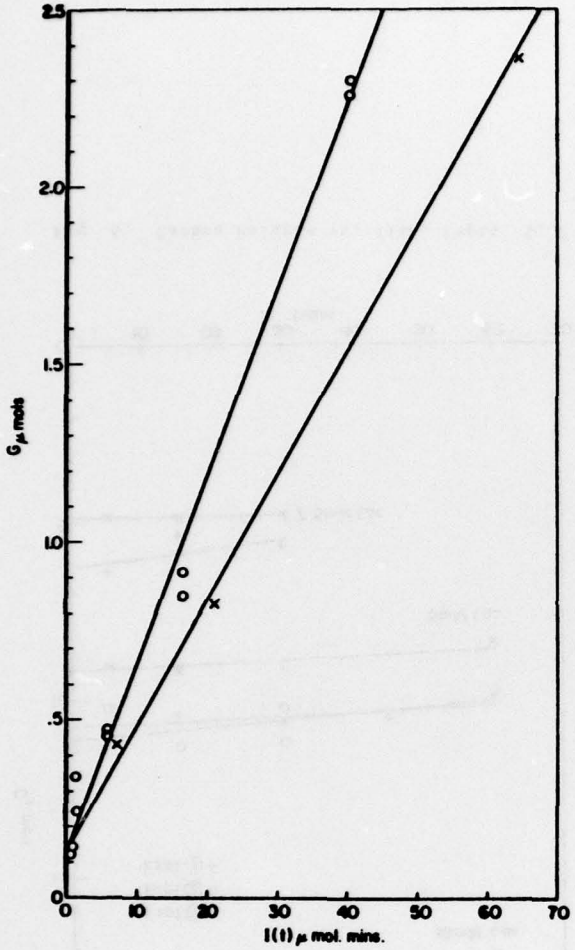


Fig. 5B. Glucose vs. Integrated Pyruvate, Expt. 2

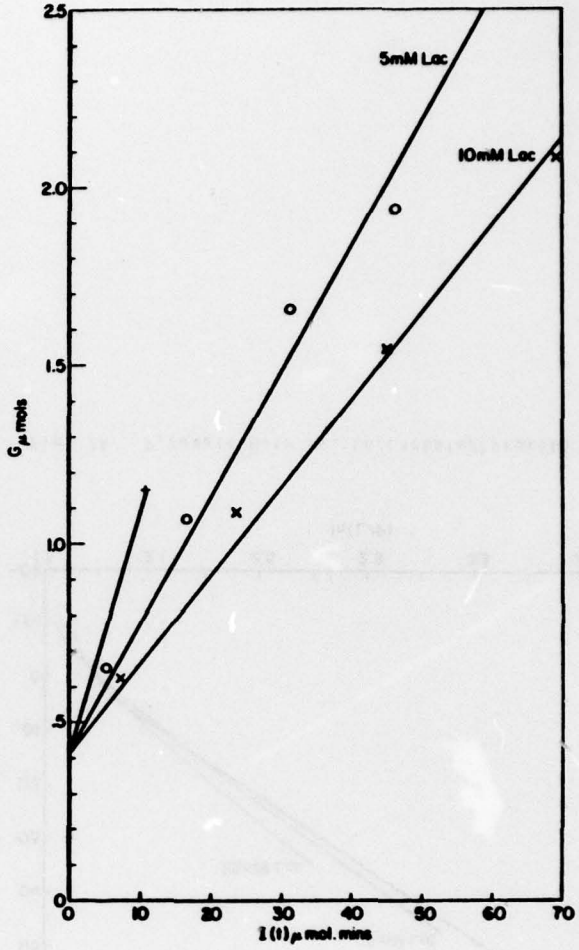


Fig. 5C. Glucose vs. Integrated Pyruvate, Expt. 3

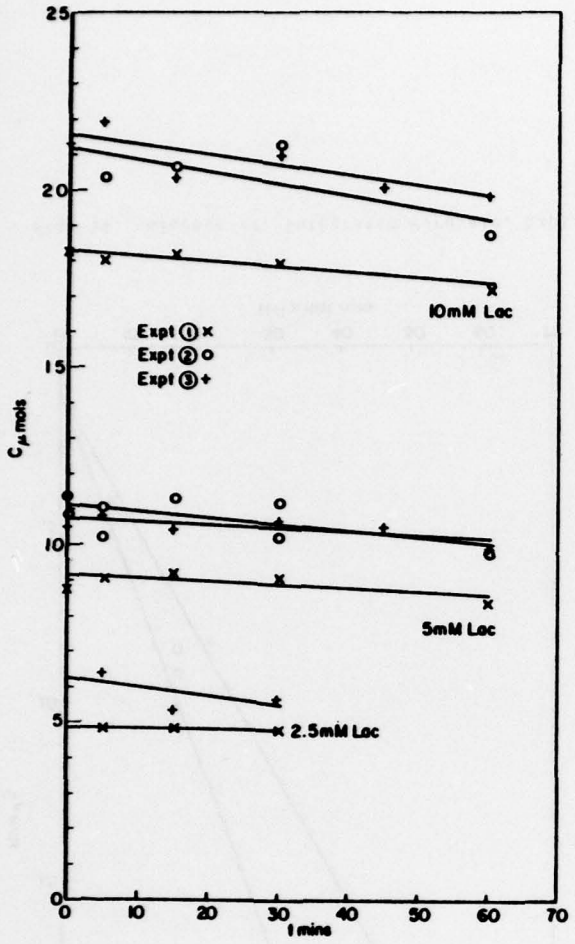


Fig. 6. Carbon Balance vs. Time, Expts. 1, 2 and 3

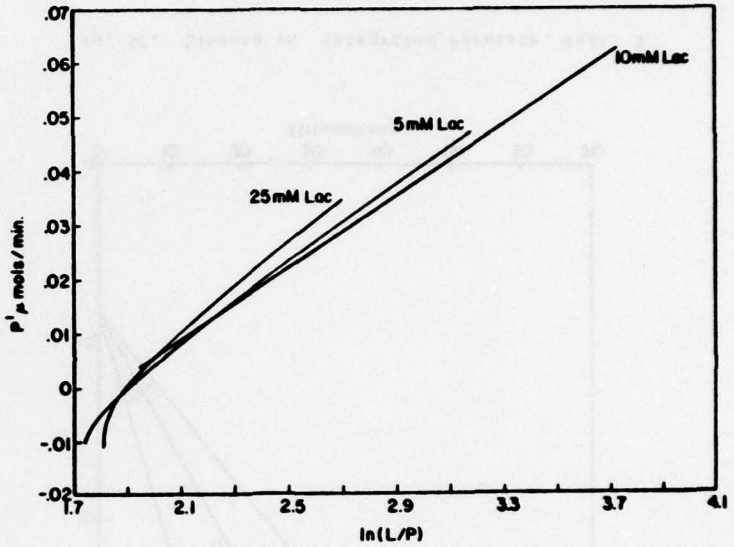


Fig. 7A. Pyruvate Rate vs. Ln Lactate/Pyruvate Ratio, Expt. 1

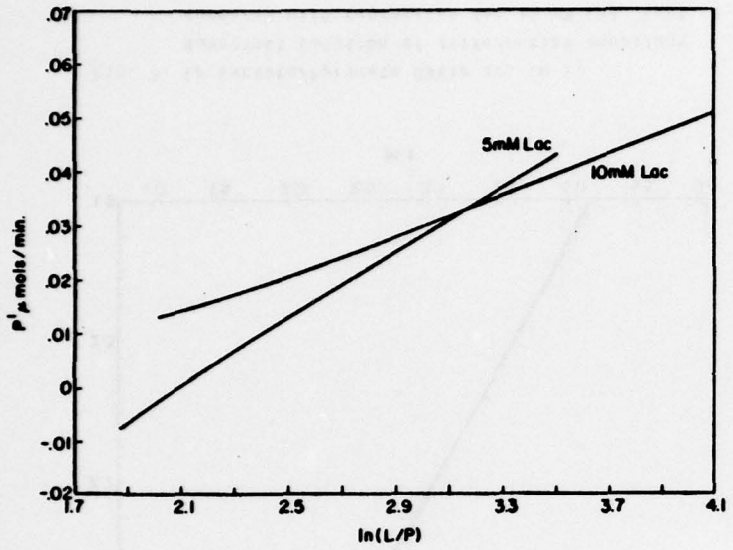


Fig. 7B. Pyruvate Rate vs. Ln Lactate/Pyruvate Ratio, Expt. 2

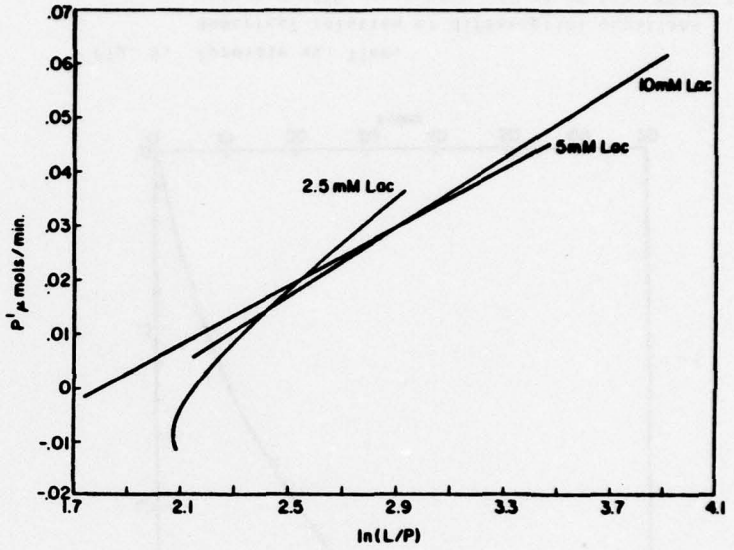


Fig. 7C. Pyruvate Rate vs. Ln Lactate/Pyruvate Ratio, Expt. 3

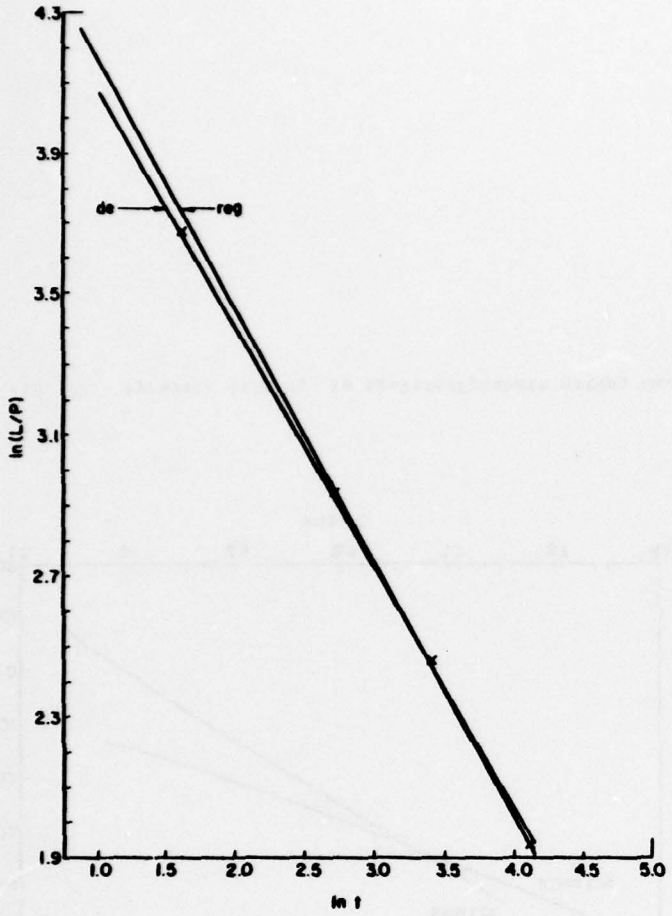


Fig. 8. Ln Lactate/Pyruvate Ratio vs. Ln t.
Numerical solution of differential equations
compared with regression for 10 mM Lac, Expt. 1

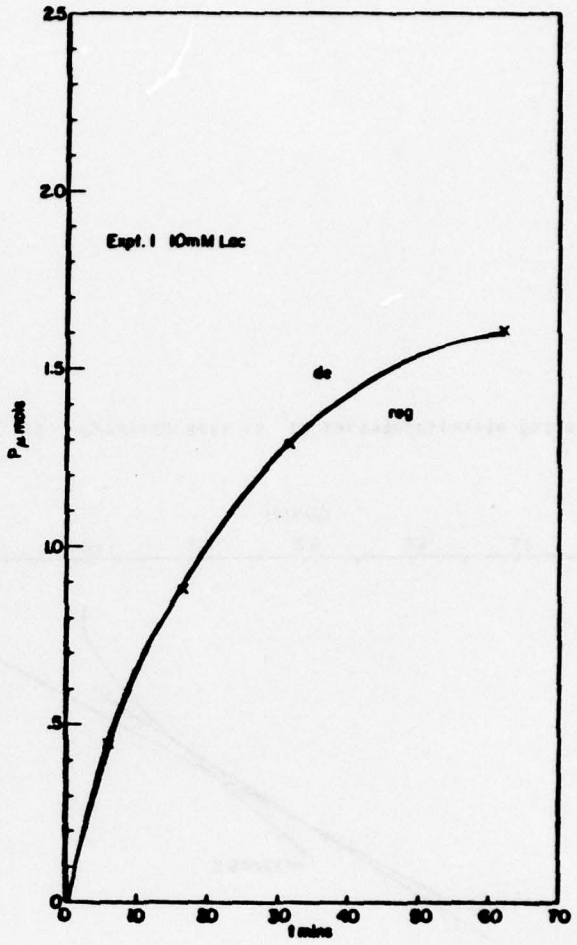


Fig. 9. Pyruvate vs. Time.
Numerical solution of differential equations
compared with regression for 10 mM Lac, Expt. 1

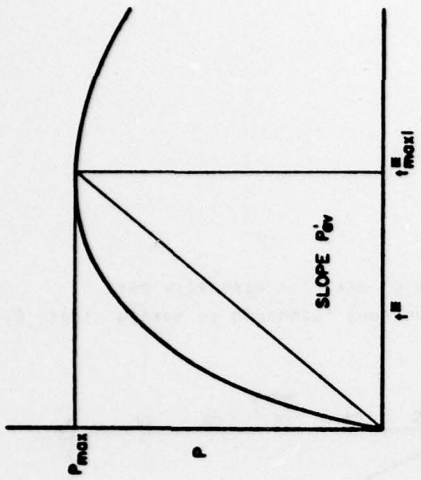


Fig. 11. Illustration of the constants t_{max1}^{*}, P_{max} and P'_{av} for the pyruvate curve.

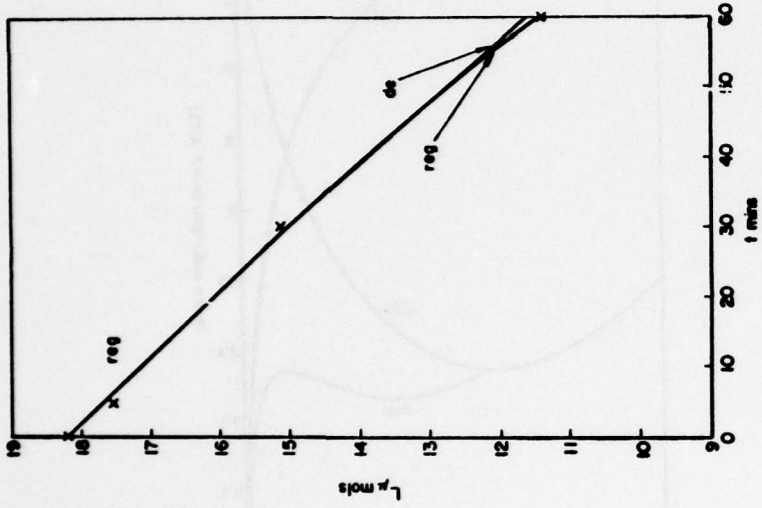


Fig. 10. Lactate vs. Time.
Numerical solution of differential equations compared with regression for 10 mM Lac, Expt. 1.

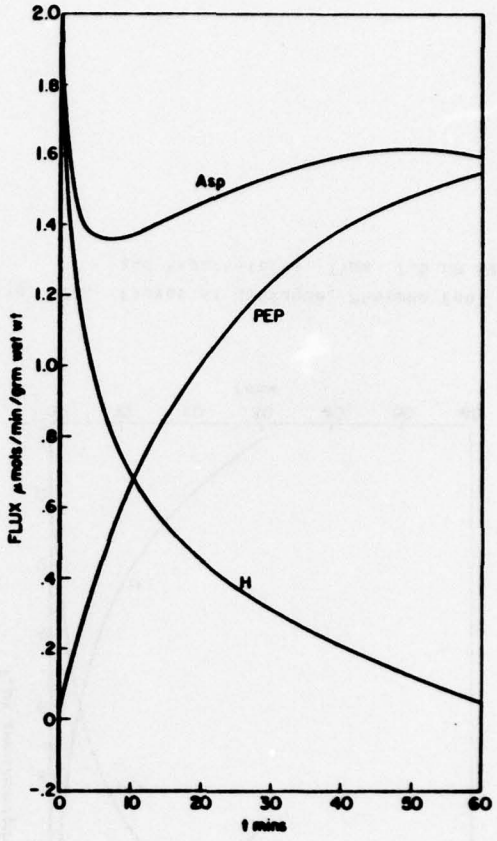


Fig. 12A. Fluxes of Hydrogen, Phospho Enol Pyruvate and Aspartate vs. Time 10 mM Lac, Expt. 1

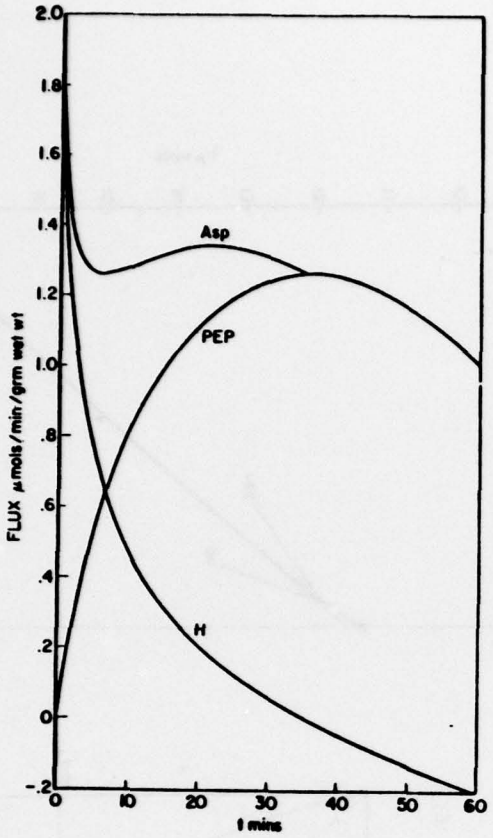


Fig. 12B. Fluxes of Hydrogen, Phospho Enol Pyruvate and Aspartate vs. Time 5 mM Lac, Expt. 1

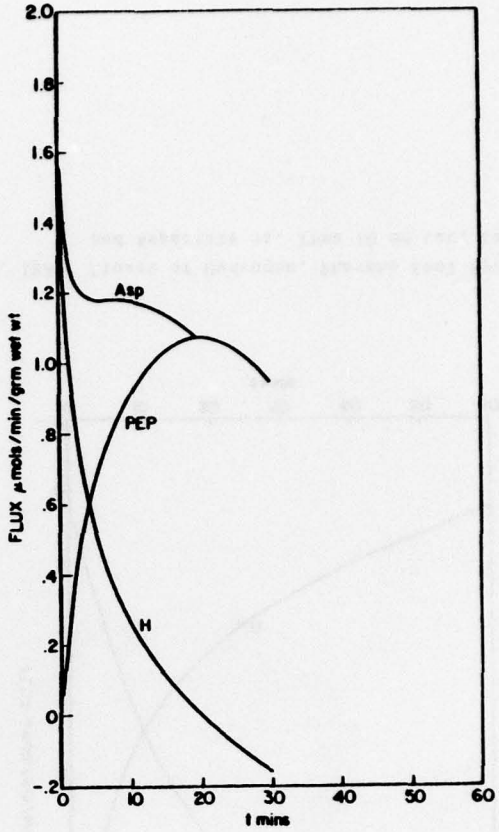


Fig. 12C. Fluxes of Hydrogen, Phospho Enol Pyruvate and Aspartate vs. Time 2.5 mM Lac, Expt. 1

LEGEND OF FIGURES

Fig. 1A Pyruvate vs. Time, Expt. 1

Fig. 1B Pyruvate vs. Time, Expt. 2

Fig. 1C Pyruvate vs. Time, Expt. 3

Fig. 2 Glucose minus Initial Glucose vs. Time, Expt. 1

Fig. 3 Lactate vs. Time, Expts. 1, 2, and 3

Fig. 4 Ln Lactate/Pyruvate Ratio vs. Ln t, Expt. 1

Fig. 5A Glucose vs. Integrated Pyruvate, Expt. 1

Fig. 5B Glucose vs. Integrated Pyruvate, Expt. 2

Fig. 5C Glucose vs. Integrated Pyruvate, Expt. 3

Fig. 6 Carbon Balance vs. Time, Expts. 1, 2, and 3

Fig. 7A Pyruvate Rate vs. Ln Lactate/Pyruvate Ratio, Expt. 1

Fig. 7B Pyruvate Rate vs. Ln Lactate/Pyruvate Ratio, Expt. 2

Fig. 7C Pyruvate Rate vs. Ln Lactate/Pyruvate Ratio, Expt. 3

Fig. 8 Ln Lactate/Pyruvate Ratio vs. Ln t. Numerical solution of differential equations compared with regression for 10mM Lac, Expt. 1

Fig. 9 Pyruvate vs. Time. Numerical solution of differential equations compared with regression for 10mM Lac, Expt. 1

Fig. 10 Lactate vs. Time. Numerical solution of differential equations compared with regression for 10mM Lac, Expt. 1

Fig. 11 Illustration of the constants t_{max1}*, P_{max} and P'_{av} for the pyruvate curve

Fig. 12A Fluxes of Hydrogen, Phospho Enol Pyruvate and Aspartate vs. Time 10mM Lac, Expt. 1

Fig. 12B Fluxes of Hydrogen, Phospho Enol Pyruvate and Aspartate vs. Time 5mM Lac, Expt. 1

Fig. 12C Fluxes of Hydrogen, Phospho Enol Pyruvate and Aspartate vs. Time 2.5mM Lac, Expt. 1

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER: Tech. Report. #146, Series 2
2. GOVT ACCESSION NO.:
3. RECIPIENT'S CATALOG NUMBER:
4. TITLE (and Subtitle): LIVER REDOX RESISTANCE, A Dynamic Model of Gluconeogenic Lactate Metabolism
5. TYPE OF REPORT & PERIOD COVERED: Technical Report
6. PERFORMING ORG. REPORT NUMBER:
7. AUTHOR(s): Alan T. James
8. CONTRACT OR GRANT NUMBER(s): N00014-75-C-0453
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Department of Statistics, Princeton University, Princeton, N. J. 08544
10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS:
11. CONTROLLING OFFICE NAME AND ADDRESS: Office of Naval Research (Code 436), Arlington, Virginia 22217
12. REPORT DATE: March 1979
13. NUMBER OF PAGES: 37
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office):
15. SECURITY CLASS. (of this report): UNCLASSIFIED
15a. DECLASSIFICATION/DOWNGRADING SCHEDULE:
16. DISTRIBUTION STATEMENT (of this Report): approved for public release; distribution unlimited.
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report):
18. SUPPLEMENTARY NOTES: Professor James is on leave from The University of Adelaide, Adelaide, South Australia.
19. KEY WORDS (Continue on reverse side if necessary and identify by block number): Redox resistance.

20. ABSTRACT-For isolated hepatocytes from starved rats metabolising lactate, the time curves of pyruvate and glucose produced and lactate remaining are fitted with elementary mathematical functions by simple statistical procedures. The fitted functions are very close approximations to the solutions of differential equations which express the following model: (i) The rate rate of net pyruvate production, which involves hydrogen disposal, is proportional to the rise of cytosolic redox potential. (ii) Glucose is produced at a rate proportional to the concentration of pyruvate, the initial substrate in the chain of reactions. (iii) The lactate remaining is that part not transformed into other substances or catabolised. Since the rise of redox potential is measured in volts and the net hydrogen flux from the lactate can be expressed in amps of electron flow involved, the ratio in ohms is called a redox resistance.

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE S/N 0102-LF-014-6601

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)